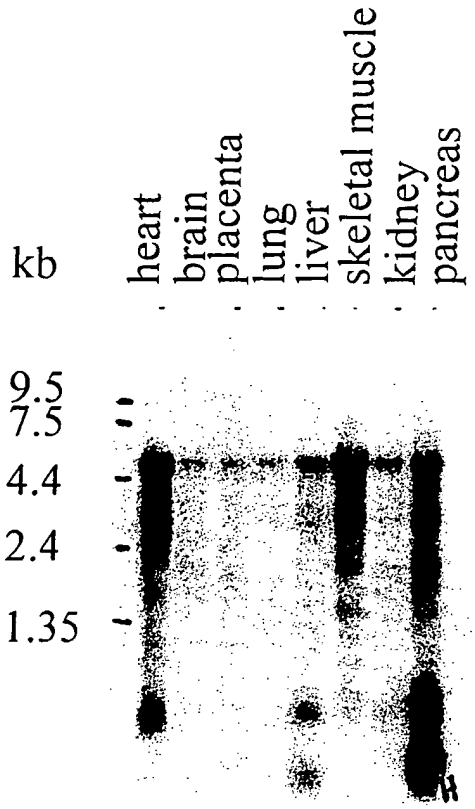


FIG. 1A

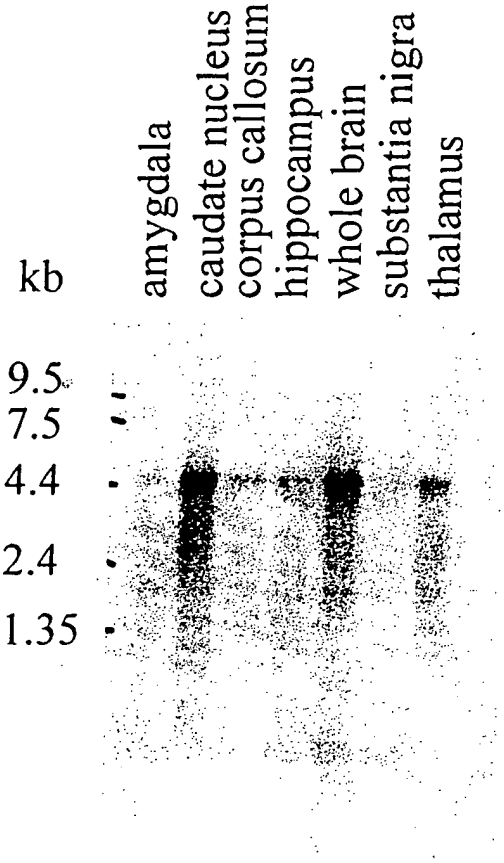


FIG. 1B

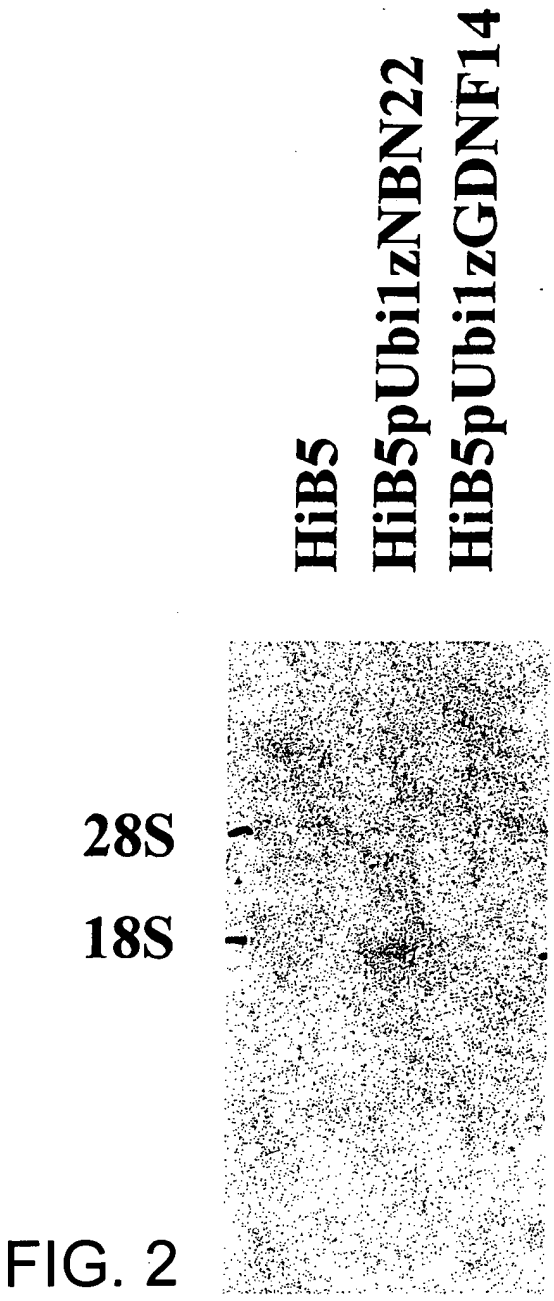


FIG. 2

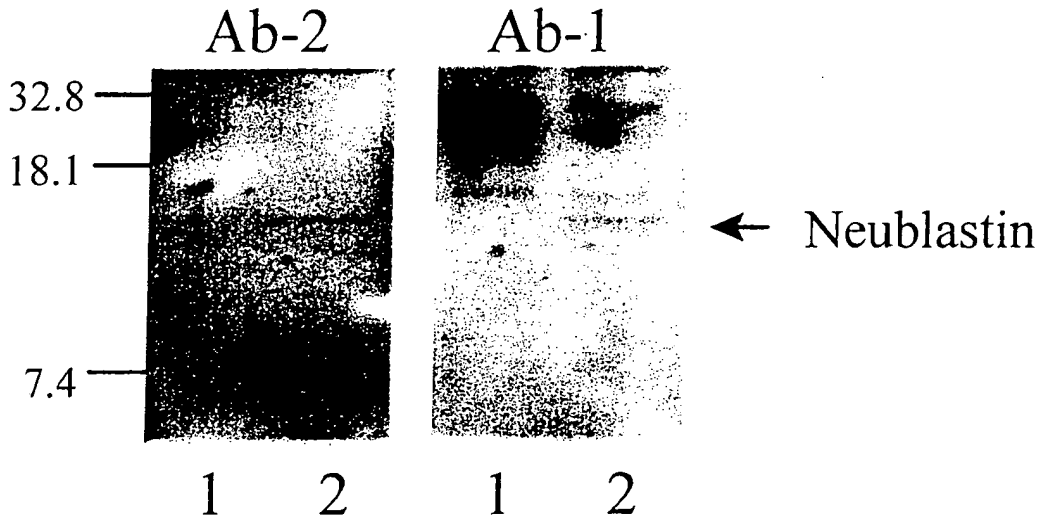


FIG. 3

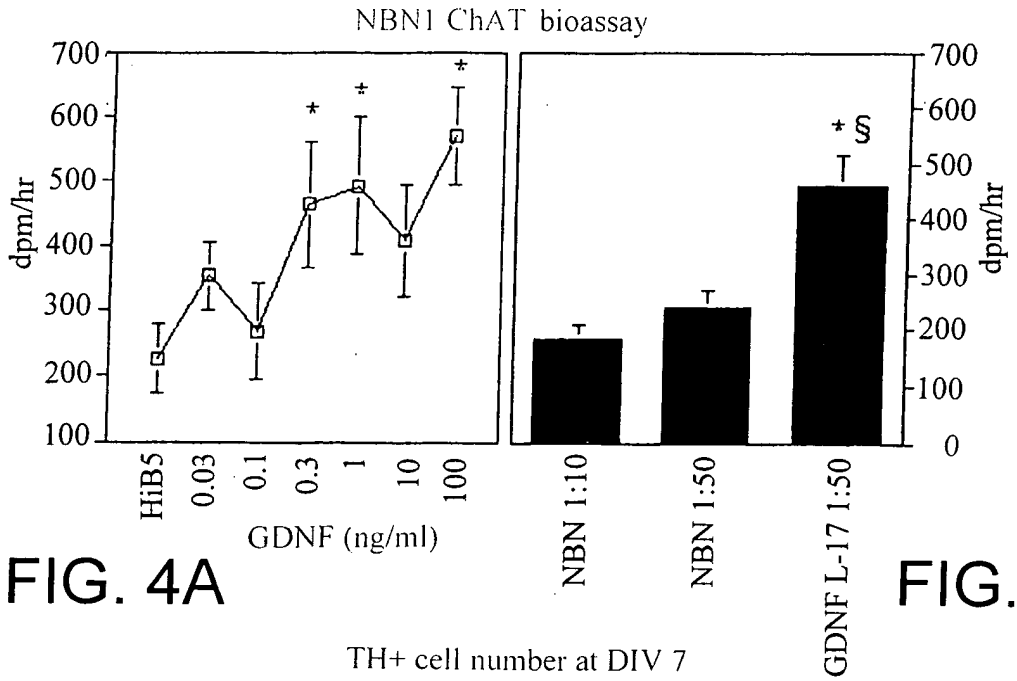


FIG. 4A

FIG. 4B

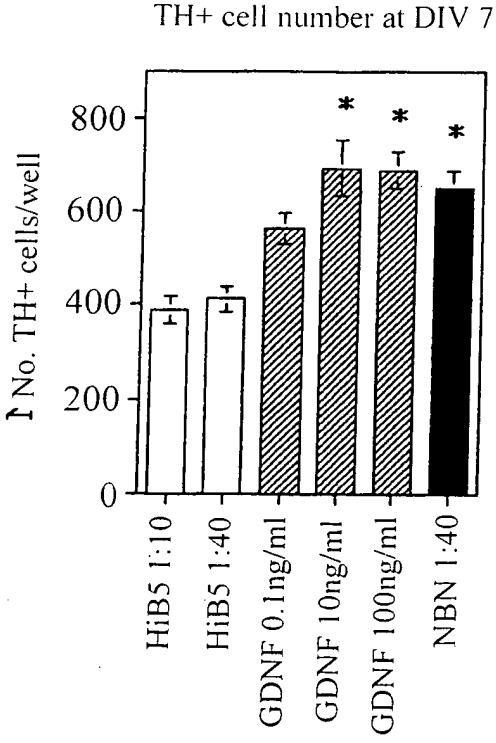


FIG. 4C

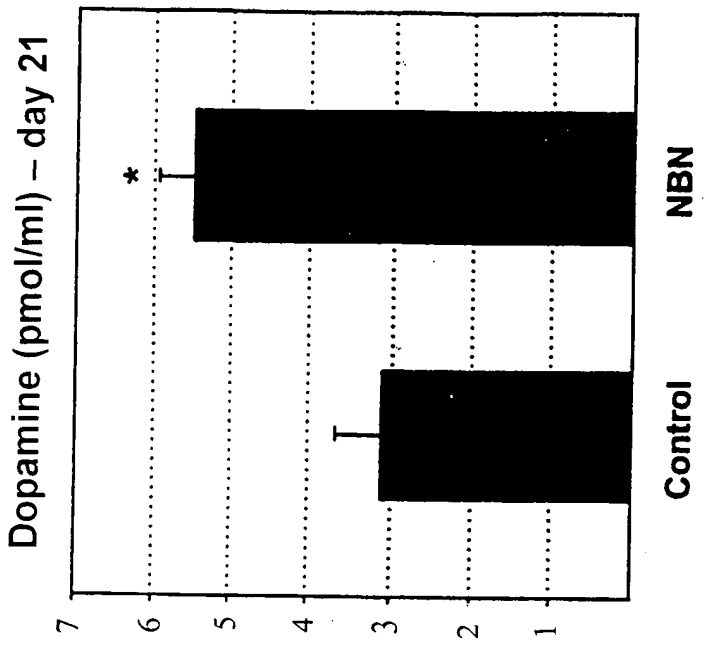

Dopamine (pmol/ml) – day 21
7
6
5
4
3
2
1
*
Control
NBN

FIG. 5B

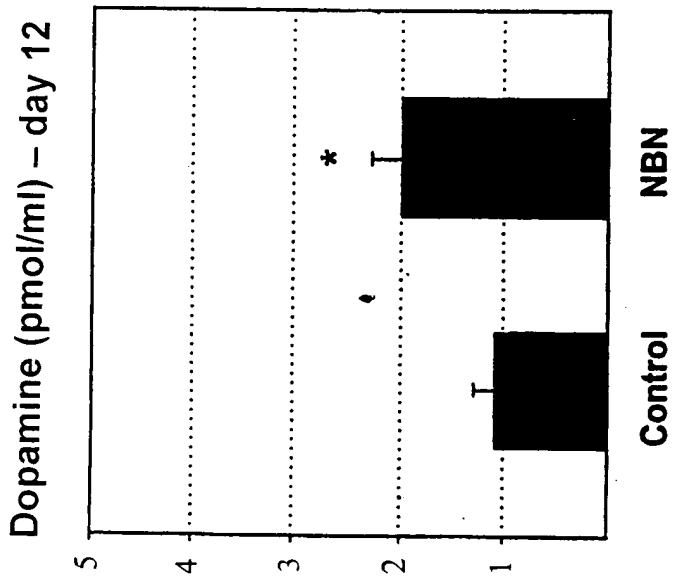

Dopamine (pmol/ml) – day 12
5
4
3
2
1
*
Control
NBN

FIG. 5A

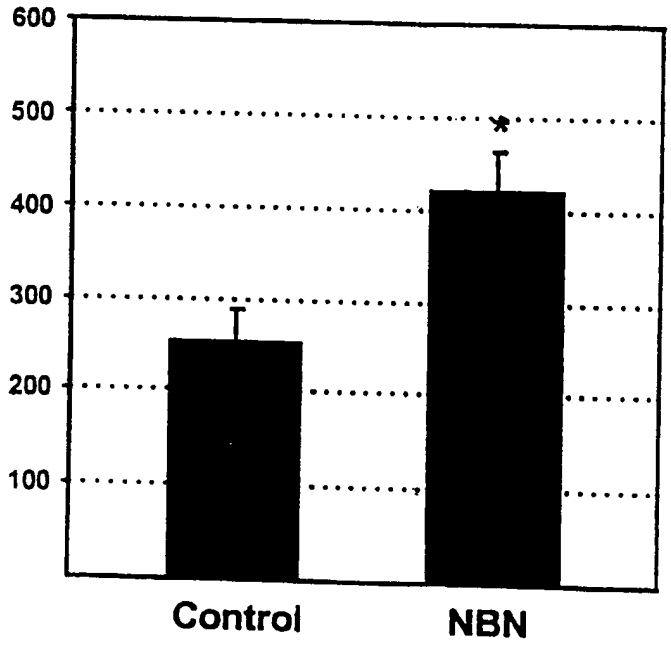


FIG. 5C

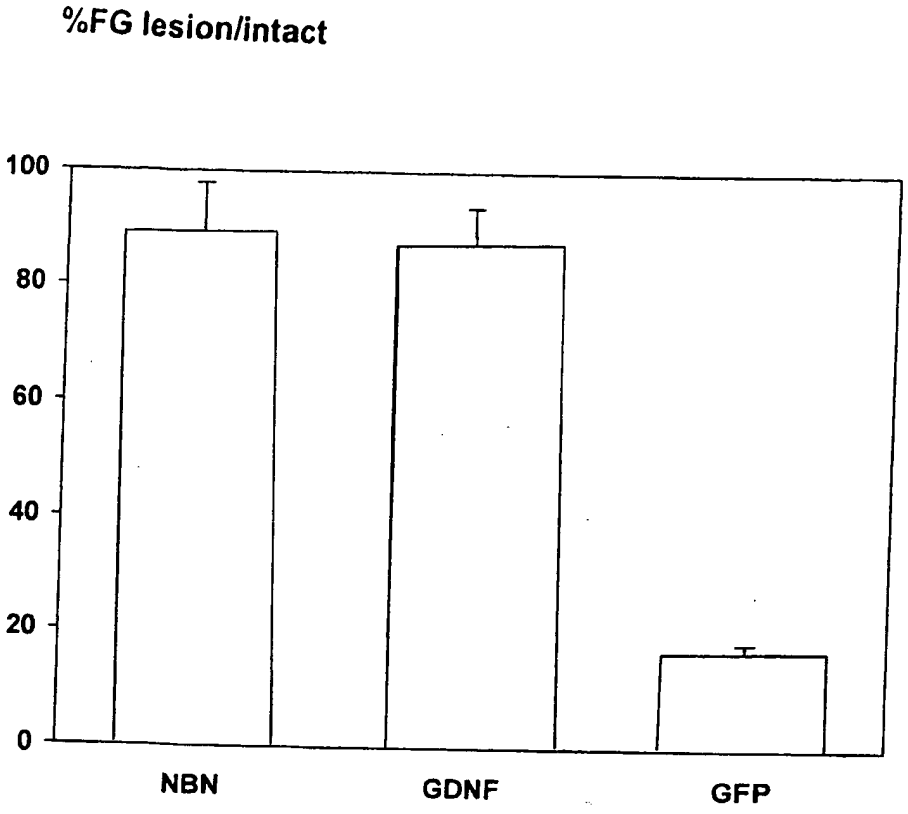


FIG. 6

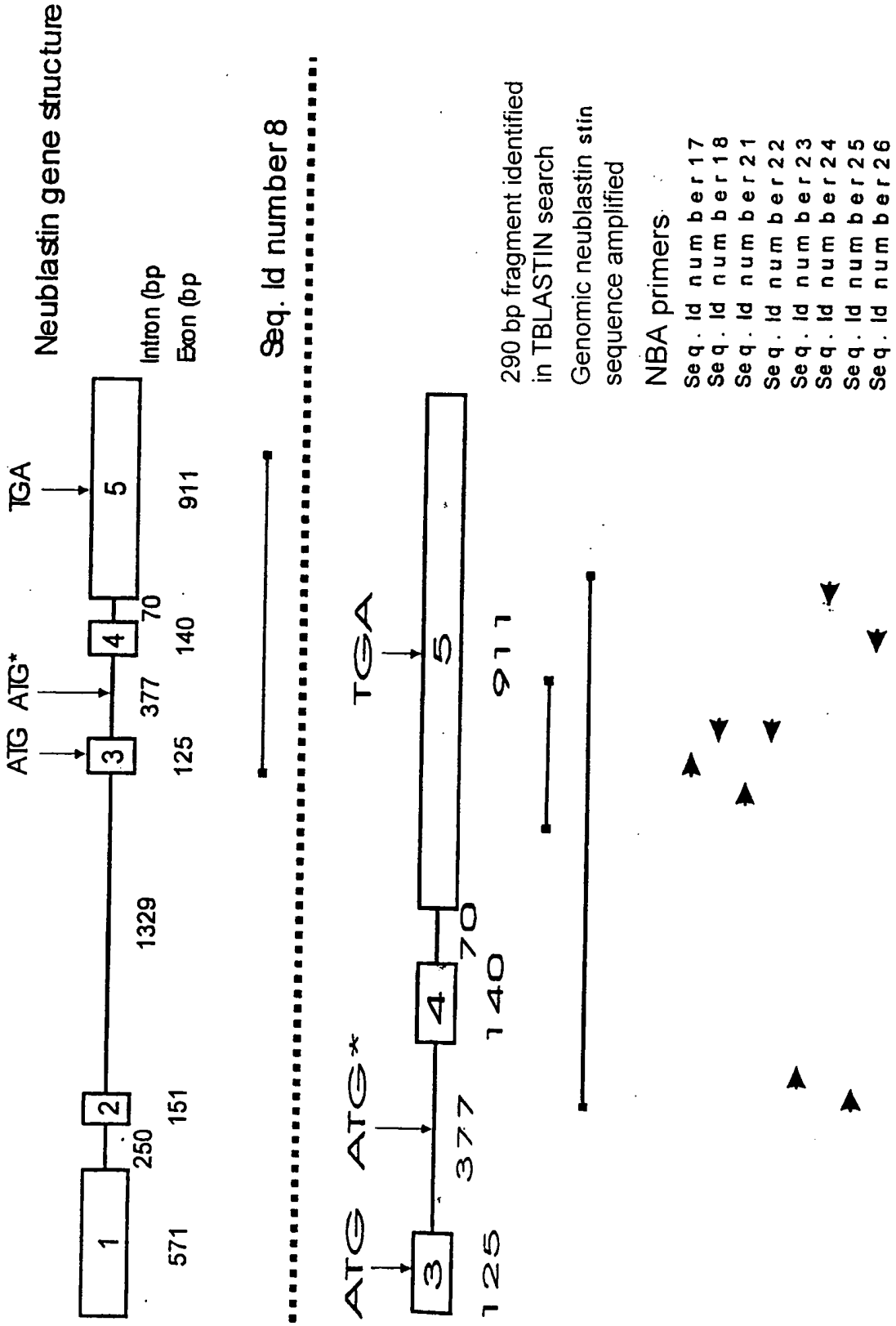

Neublastin gene structure
Intron (bp
Exon (bp
TGA
ATG ATG*
911
140
70
377
125
1329
151
250
571
Seq. Id number 8
290 bp fragment identified in TBLASTIN search
Genomic neublastin stin sequence amplified
NBA primers
Seq. Id number 17
Seq. Id number 18
Seq. Id number 21
Seq. Id number 22
Seq. Id number 23
Seq. Id number 24
Seq. Id number 25
Seq. Id number 26

FIG. 7

Alignment of Neublastin primers used in Rapid-Screen with homologous regions in other GDNF ligands

5'-C **CTG GCC AGC CTA CTG GG**-3'	**SEQ ID No 17**
G **CTG GCC CGG CTG CAG GG**	persephin
G **CTG CGA CGA CTG CGC CA**	neurturin
A **TTG AAA AAC TTA TCC AG**	GDNF

5'-**AA GGA GAC CGC TTC GTA GCG**-3'	**SEQ ID No 18**
TA GGC CAC GTC GGT GTA GCG	persephin
AA GGA CAC CTC GTC CTC GTA GGC	neurturin
AA CGA CAG GTC ATC ATC AAA GGC	GDNF

conserved nucleotides shown in **bold**

FIG. 8

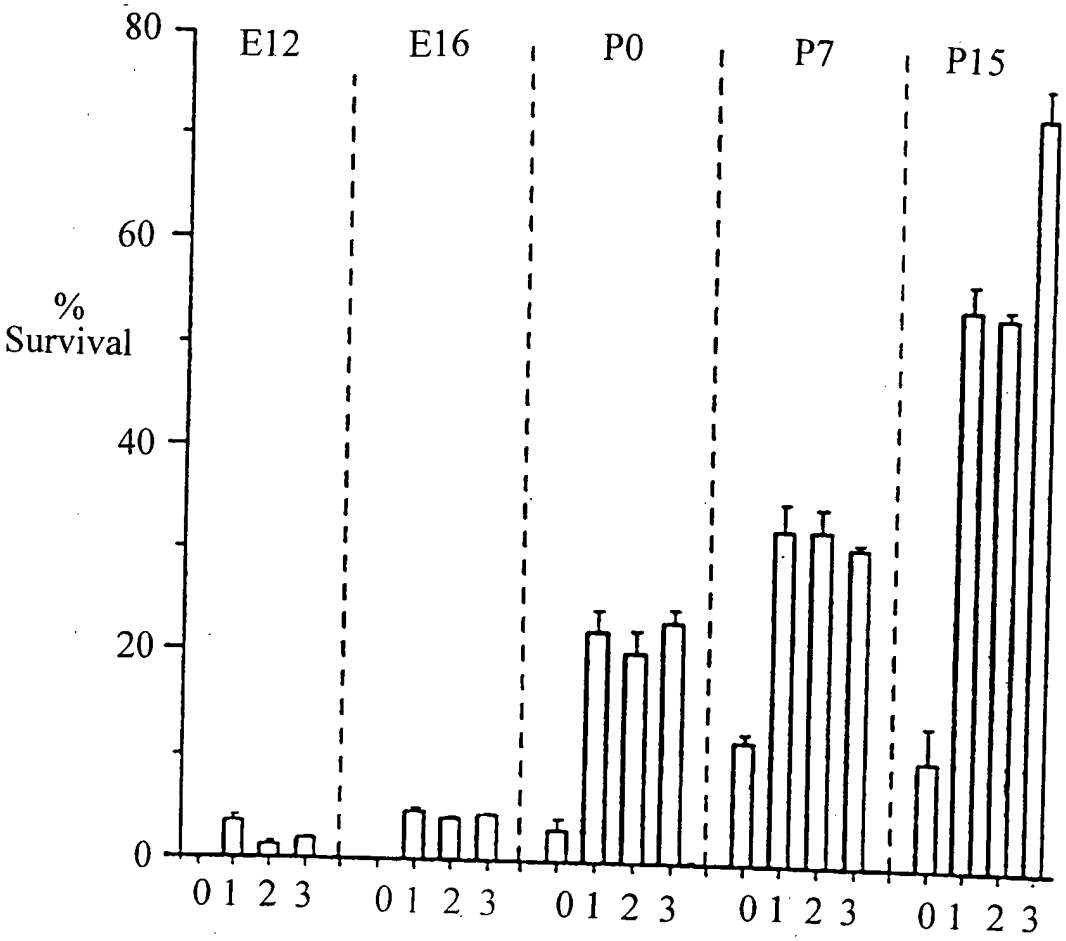

80
60
40
20
0
% Survival
E12
E16
P0
P7
P15
0 1 2 3
0 1 2 3
0 1 2 3
0 1 2 3
0 1 2 3

FIG. 9

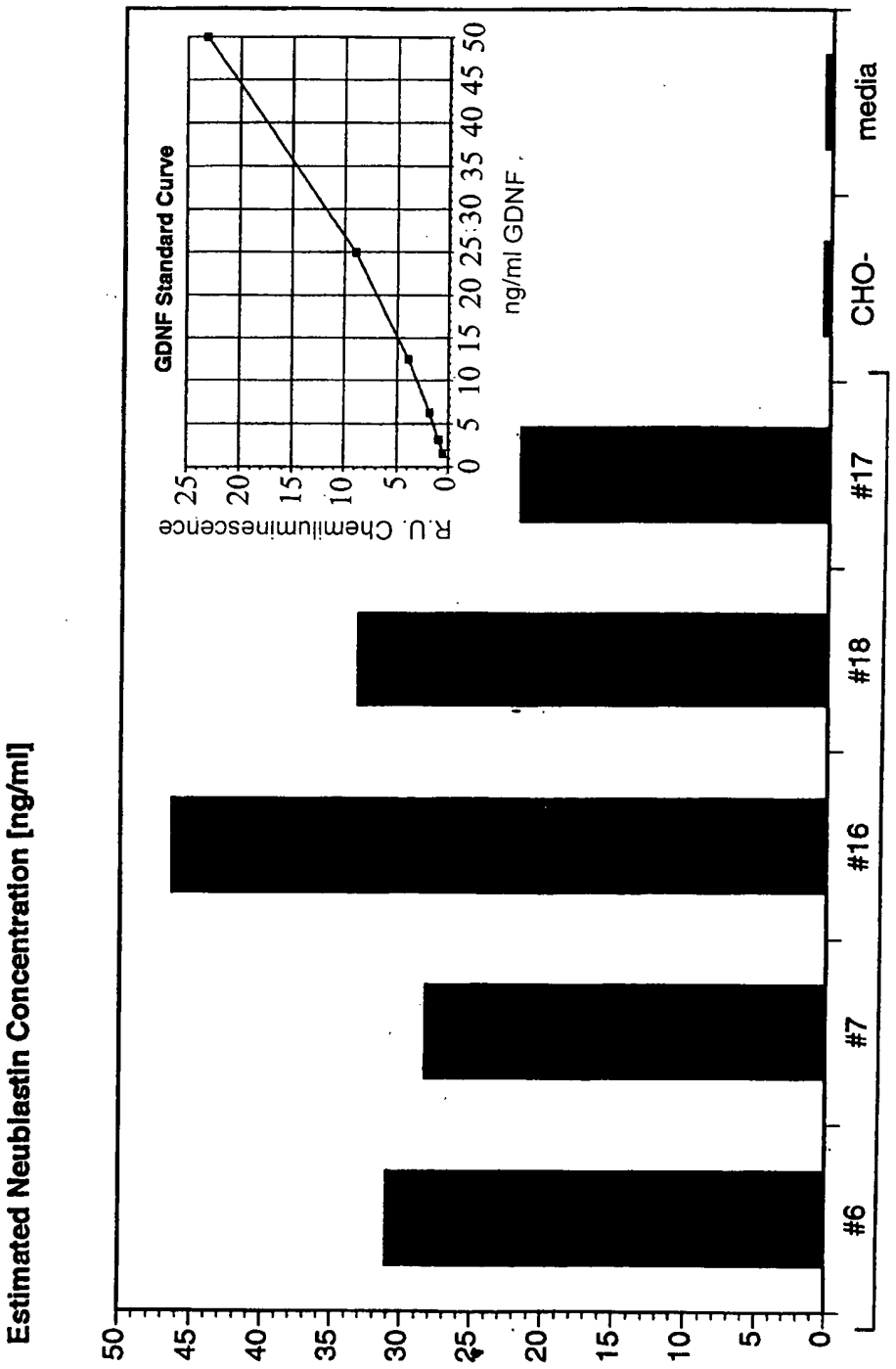

Estimated Neublastin Concentration [ng/ml]
50
45
40
35
30
25
20
15
10
5
0
#6
#7
#16
#18
#17
CHO-
media
CHO Neublastin Clones
GDNF Standard Curve
R.L.U. Chemiluminescence
25
20
15
10
5
0
0 5 10 15 20 25 30 35 40 45 50
ng/ml GDNF

FIG. 10

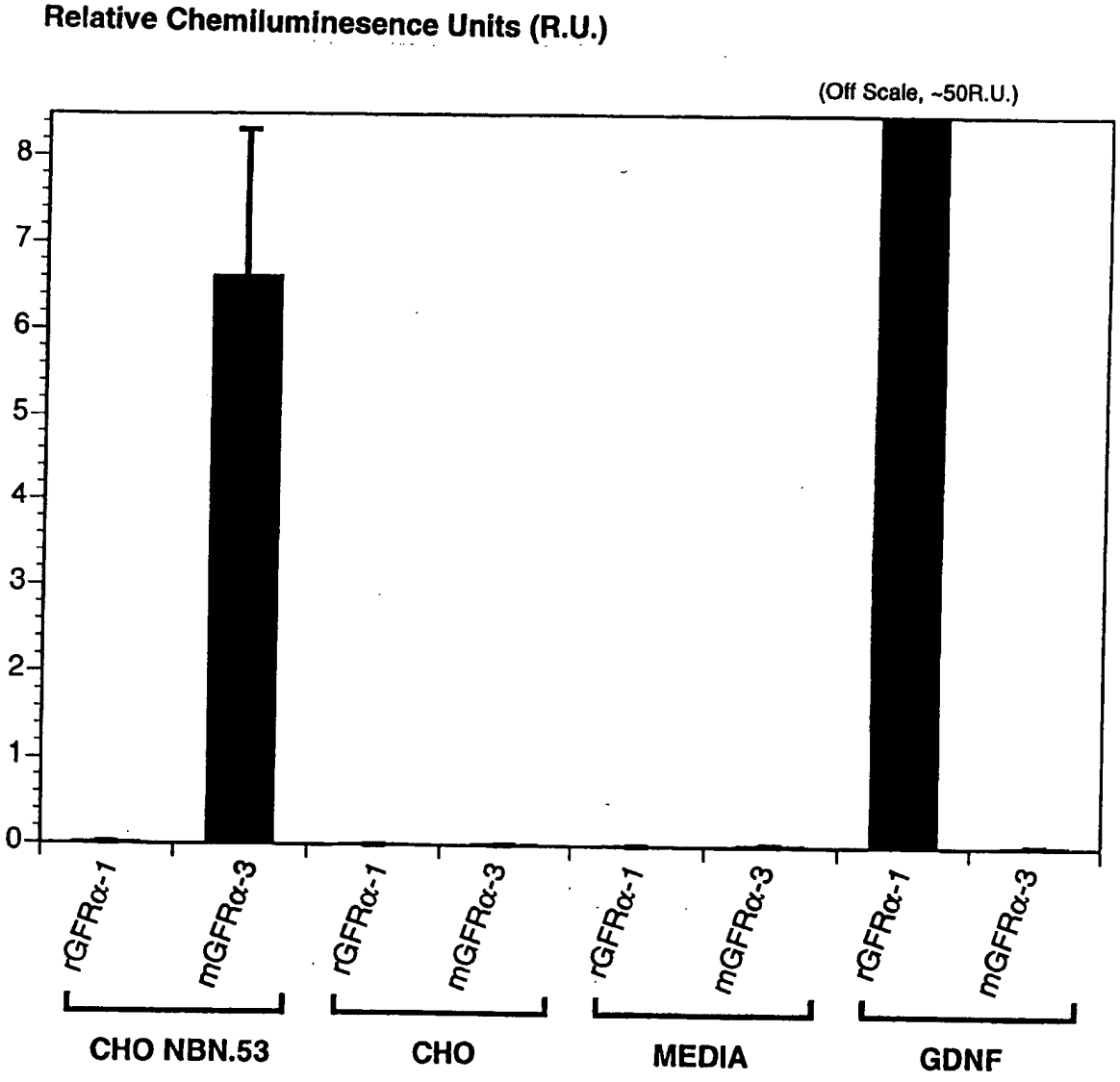
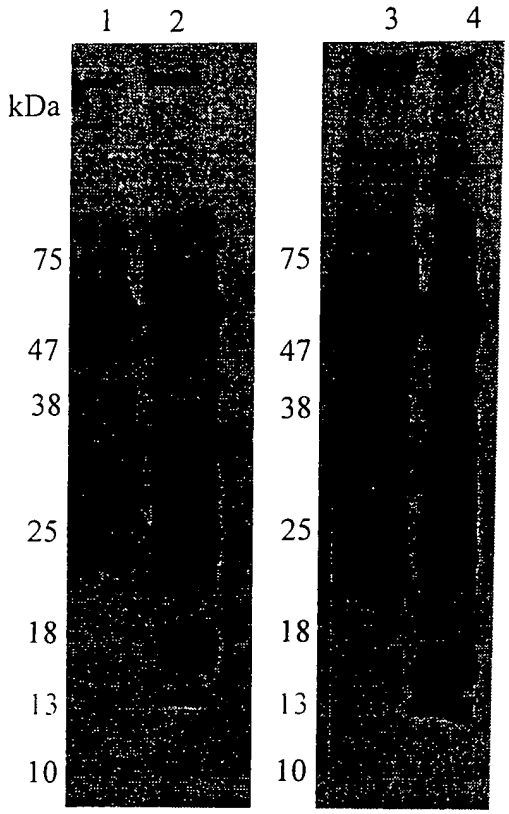
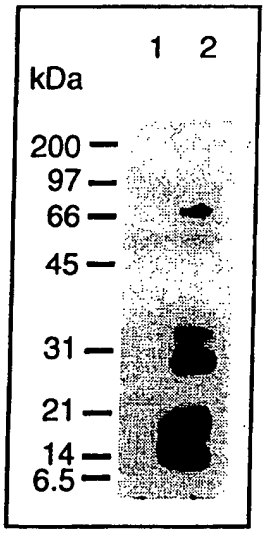

Relative Chemiluminesence Units (R.U.)
(Off Scale, ~50R.U.)
8
7
6
5
4
3
2
1
0
rGFRα-1
mGFRα-3
rGFRα-1
mGFRα-3
rGFRα-1
mGFRα-3
rGFRα-1
mGFRα-3
CHO NBN.53
CHO
MEDIA
GDNF

FIG. 11



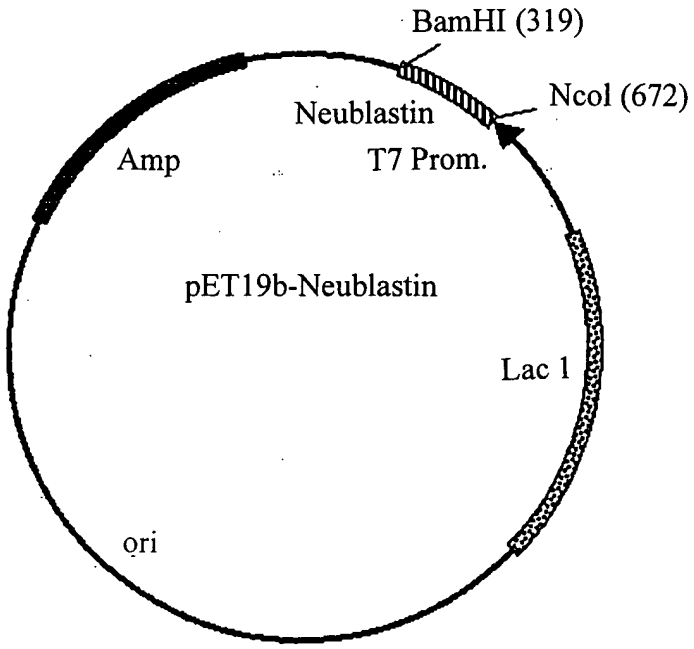
1. Control medium stained with R30 anti-peptide antibody
2. Neublastin containing conditioned medium stained with R30 anti-peptide antibody
3. Control medium stained with R31 anti-peptide antibody
4. Neublastin containing conditioned medium stained with R31 anti-peptide antibody

FIG. 12



Extraction of neublastin by affinity-binding on RETL3-Ig
Lane 1: bound from CHO control conditioned media
Lane 2: bound from neublastin overexpressing CHO conditioned media

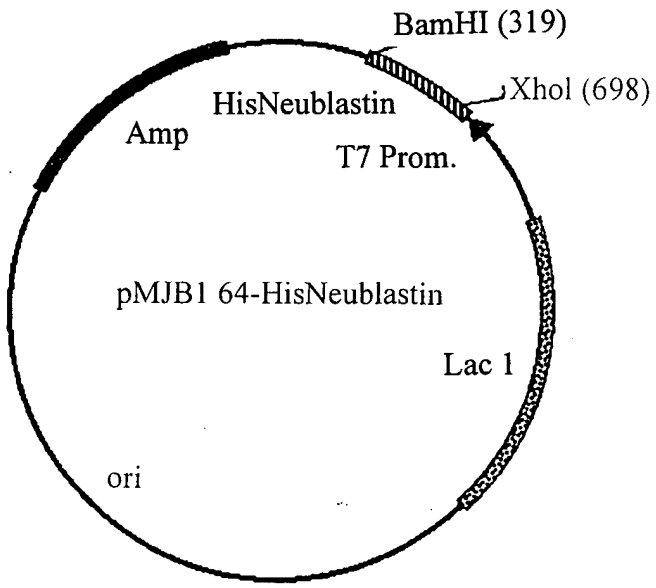
FIG. 13



Neublastin Syngene

```
      NcoI (318)
316  TACCATGGCT GGAGGACCGG GATCTCGTGC TCGTGCAGCA GGAGCACGTG GCTGTCGTCT
     ATCCTACCGA CCTCCTGGCC CTAGAGCACG AGCACGTCGT CCTCGTGCAC CGACAGCAGA
       1▶ M A   G G P   G S R A   R A A   G A R   G C R L

376  GCGTTCTCAA CTAGTGCCGG TGCGTGCACT CGGACTGGGA CACCGTTCCG ACGAACTAGT
     CGCAAGAGTT GATCACGGCC ACGCACGTGA GCCTGACCCT GTGGCAAGGC TGCTTGATCA
      19▶ R S Q   L V P   V R A L   G L G   H R S   D E L V

436  ACGTTTTCGT TTTTGTTCAG GATCTTGTCG TCGTGCACGT TCTCCGCATG ATCTATCTCT
     TGCAAAAGCA AAAACAAGTC CTAGAACAGC AGCACGTGCA AGAGGCGTAC TAGATAGAGA
      39▶ R F R   F C S   G S C R   R A R   S P H   D L S L

496  AGCATCTCTA CTAGGAGCCG GAGCACTAAG ACCGCCGCCG GGATCTAGAC CTGTATCTCA
     TCGTAGAGAT GATCCTCGGC CTCGTGATTC TGGCGGCGGC CCTAGATCTG GACATAGAGT
      59▶ A S L   L G A   G A L R   P P P   G S R   P V S Q

556  ACCTTGTTGT AGACCTACTA GATACGAAGC AGTATCTTTC ATGGACGTAA ACTCTACATG
     TGGAACAACA TCTGGATGAT CTATGCTTCG TCATAGAAAG TACCTGCATT TGAGATGTAC
      79▶ P C C   R P T   R Y E A   V S F   M D V   N S T W

                                                          BamHI (671)
616  GAGAACCGTA GATAGACTAT CTGCAACCGC ATGTGGCTGT CTAGGATGAT AATAGGGATC
     CTCTTGGCAT CTATCTGATA GACGTTGGCG TACACCGACA GATCCTACTA TTATCCCTAG
      99▶ R T V   D R L   S A T A   C G C   L G •   • •

676  CGGCT
     GCCGA
```

FIG. 14



HisNeublastin

XhoI (340)

```
301  TACCATGGGC CATCATCATC ATCATCATCA TCATCATCAC TCGAGCGGCC ATATCGACGA
     ATCCTACCCG GTAGTAGTAG TAGTAGTAGT AGTAGTAGTG AGCTCGCCGG TATAGCTGCT
       1▶ M  G   H  H  H   H  H  H  H   H  H  H   S  S  G   H  I  D  D

361  CGACGACAAG GCTGGAGGAC CGGGATCTCG TGCTCGTGCA GCAGGAGCAC GTGGCTGTCG
     GCTGCTGTTC CGACCTCCTG GCCCTAGAGC ACGAGCACGT CGTCCTCGTG CACCGACAGC
  19▶  D  D  K   A  G  G   P  G  S  R   A  R  A   A  G  A   R  G  C  R

421  TCTGCGTTCT CAACTAGTGC CGGTGCGTGC ACTCGGACTG GGACACCGTT CCGACGAACT
     AGACGCAAGA GTTGATCACG GCCACGCACG TGAGCCTGAC CCTGTGGCAA GGCTGCTTGA
  39▶  L  R  S   Q  L  V   P  V  R  A   L  G  L   G  H  R   S  D  E  L

481  AGTACGTTTT CGTTTTTGTT CAGGATCTTG TCGTCGTGCA CGTTCTCCGC ATGATCTATC
     TCATGCAAAA GCAAAAACAA GTCCTAGAAC AGCAGCACGT GCAAGAGGCG TACTAGATAG
  59▶  V  R  F   R  F  C   S  G  S  C   R  R  A   R  S  P   H  D  L  S

541  TCTAGCATCT CTACTAGGAG CCGGAGCACT AAGACCGCCG CCGGGATCTA GACCTGTATC
     AGATCGTAGA GATGATCCTC GGCCTCGTGA TTCTGGCGGC GGCCCTAGAT CTGGACATAG
  79▶  L  A  S   L  L  G   A  G  A  L   R  P  P   P  G  S   R  P  V  S

601  TCAACCTTGT TGTAGACCTA CTAGATACGA AGCAGTATCT TTCATGGACG TAAACTCTAC
     AGTTGGAACA ACATCTGGAT GATCTATGCT TCGTCATAGA AAGTACCTGC ATTTGAGATG
  99▶  Q  P  C   C  R  P   T  R  Y  E   A  V  S   F  M  D   V  N  S  T

                                                          BamHI (719)
661  ATGGAGAACC GTAGATAGAC TATCTGCAAC CGCATGTGGC TGTCTAGGAT GATAATAGGG
     TACCTCTTGG CATCTATCTG ATAGACGTTG GCGTACACCG ACAGATCCTA CTATTATCCC
 119▶  W  R  T   V  D  R   L  S  A  T   A  C  G   C  L  G   •  •

721  ATCCGGCTGC TAACAAAGCC CG
     TAGGCCGACG ATTGTTTCGG GC
```

FIG. 15

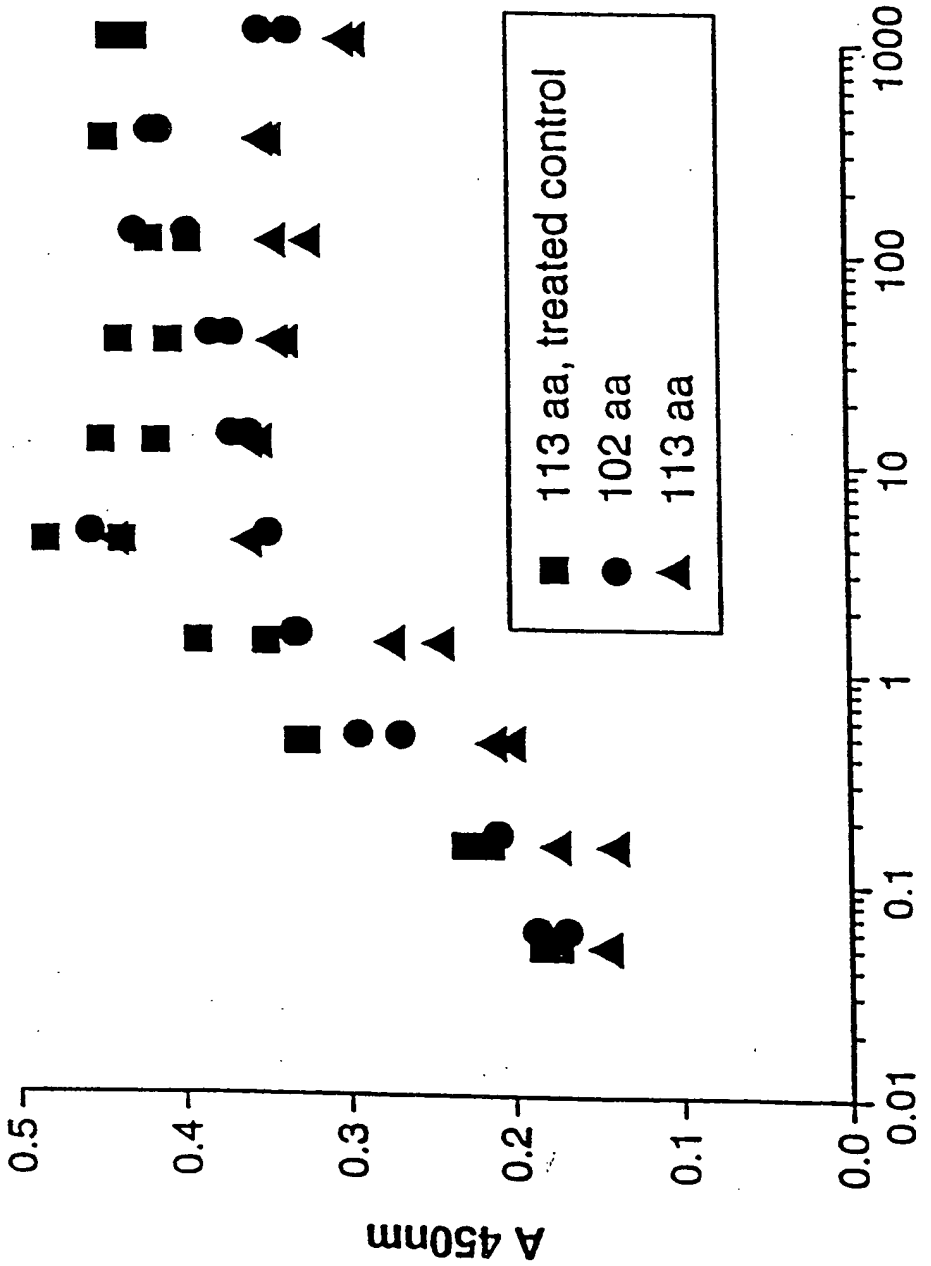

A 450nm
0.0
0.1
0.2
0.3
0.4
0.5
0.01
0.1
1
10
100
1000
[NBN] (nM)
113 aa, treated control
102 aa
113 aa

FIG. 16

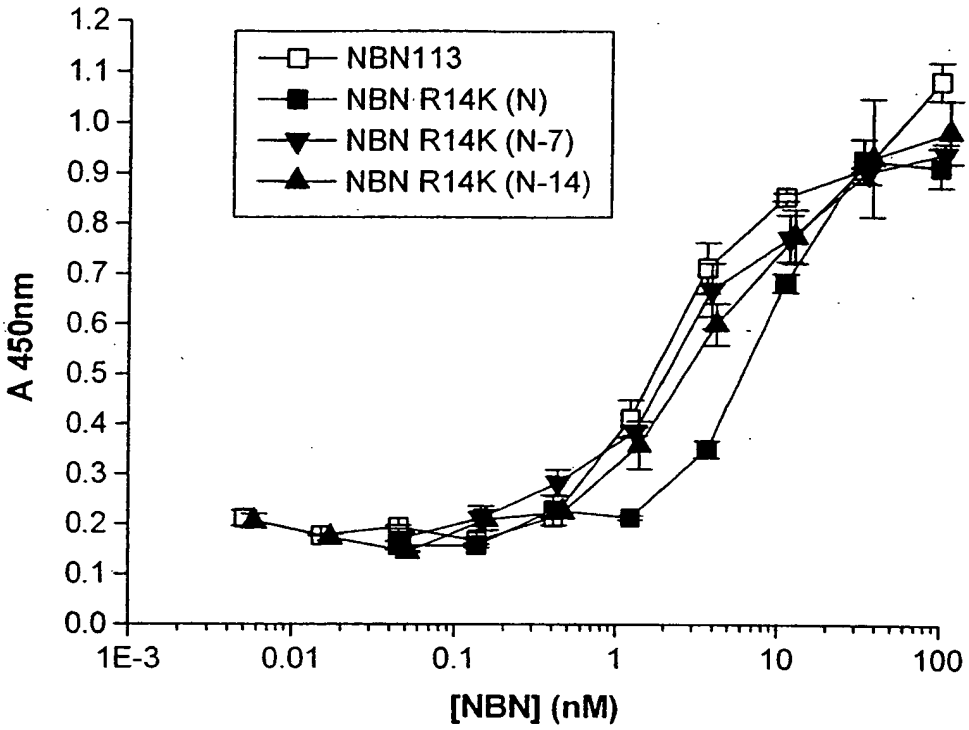

NBN113
NBN R14K (N)
NBN R14K (N-7)
NBN R14K (N-14)
A 450nm
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1E-3
0.01
0.1
1
10
100
[NBN] (nM)

FIG. 17

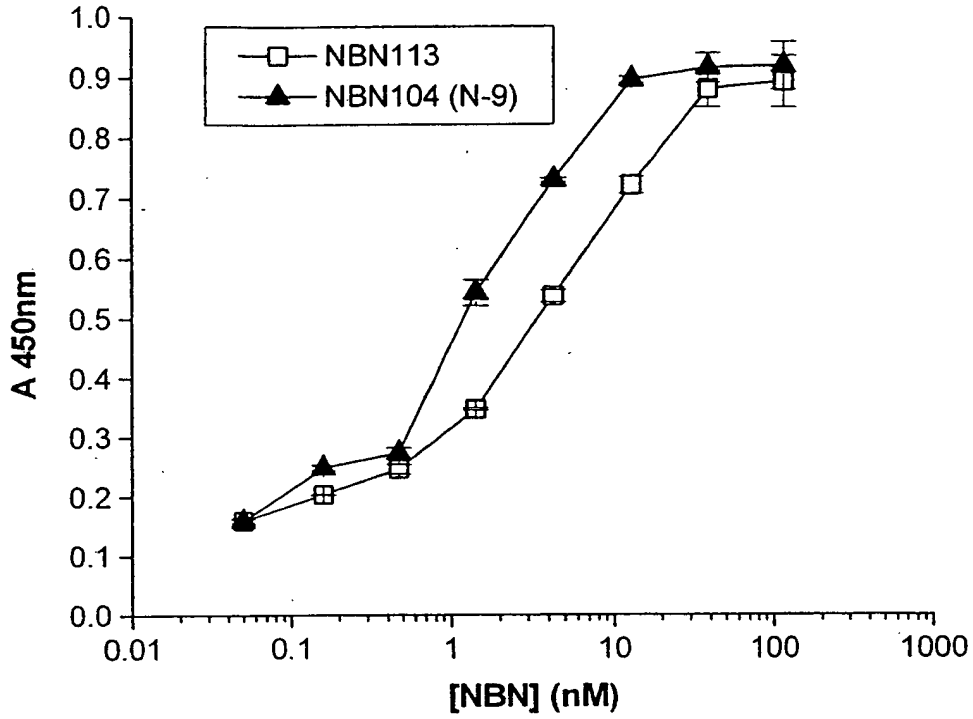

NBN113
NBN104 (N-9)
A 450nm
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.01
0.1
1
10
100
1000
[NBN] (nM)

FIG. 18